

Effects of Lasalocid or Monensin Supplementation on Digestion, Ruminal Fermentation, Blood Metabolites, and Milk Production of Lactating Dairy Cows¹

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ABSTRACT

Six ruminally fistulated midlactating multiparous Holstein cows were used in a double 3 × 3 Latin square design (35-d periods) to study the effects of lasalocid (LAS) and monensin (MON) supplemented at 24 mg/kg of dry matter on digestion, ruminal fermentation, blood metabolites, and milk production. Cows were blocked according to milk production and fed a red clover silage-based total mixed ration (17.8% crude protein) without supplementation or supplemented with LAS or MON. Daily dry matter intake, milk production, and milk fat and protein concentrations were similar among treatments and averaged 23.5 kg, 36.6 kg, 3.36%, and 3.38%, respectively. Rumen lipogenic:gluconic volatile fatty acids and NH₃-N concentration were lower, and apparent digestibility of dry matter, organic matter, crude protein, and gross energy were higher with than without ionophore supplementation. Compared with LAS, MON increased concentrations of plasma urea-N and milk urea-N, and excretion of urinary urea-N and total N. Monensin also decreased N retention and tended to reduce plasma concentration of nonessential AA in comparison with LAS. Both ionophores reduced daily fecal excretion of N by 13 g compared with the control, but MON increased daily losses of urinary N by 36 g compared with LAS. Results from this study suggest that postabsorptive metabolism of N might be altered by the type of ionophore fed.

Key words: dairy cow, ionophore, lasalocid, monensin

INTRODUCTION

A major benefit of feeding ionophores (IOP) to lactating dairy cows is the increase in efficiency of N and energy utilization resulting from the shift of rumen

VFA toward less acetate and more propionate, a reduced methane production, and a decreased breakdown of dietary protein and AA in the rumen (Ipharraguerre and Clark, 2003). Monensin (MON) and lasalocid (LAS) are 2 IOP that might act through different mechanisms on N metabolism or differ in potency. For instance, MON has been reported to be more effective than LAS at inhibiting rumen urease activity in steers (66 vs. 28% reduction; Starnes et al., 1984), whereas CP apparent digestibility was higher with LAS compared with MON in lambs (Ricke et al., 1984) or compared with a combination of MON and tylosin in steers (Zinn, 1987). Urea metabolism also appears to differ between MON and LAS. Harmon et al. (1989) compared the effects of IOP on net nutrient flux in steers fed a high-concentrate diet and observed a tendency for a decreased return of urea-N to the portal-drained viscera with MON compared with LAS and tetranosin, suggesting an inhibition of urea recycling into the gut with MON.

Ionophores form a lipophilic complex with cations and facilitate their transport across membranes. Monensin has a strong preference for sodium, whereas LAS forms complexes with a number of cations, including potassium, sodium, calcium, and magnesium (Pressman et al., 1980). Therefore, mineral metabolism can be altered by feeding IOP to beef (Starnes et al., 1984; Spears et al., 1989) and dairy cattle (Cécyre, 2001; Duffield et al., 2003).

In their review, Ipharraguerre and Clark (2003) reported production results from 6 studies with LAS and 18 studies with MON. Although many experiments have looked at the effect of MON in dairy cattle, few studies are available on the effects of LAS. Furthermore, to our knowledge, a comparison of MON and LAS in the same dairy experiment could not be found. Therefore, the objectives of the present study were to compare the effects of MON and LAS on digestion, ruminal fermentation, blood metabolites, and milk production in lactating dairy cows.

MATERIALS AND METHODS

Animals used in the current study were cared for according to the guidelines of the Canadian Council

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Table 1. Ingredients and chemical composition of the TMR (control)

Item	Amount
Ingredient, % of DM	
Mixed silage ¹	52.6
Pelleted concentrate ²	47.4
Chemical composition	
DM, %	49.7
OM, % of DM	93.9
CP, % of DM	17.8
NDF, % of DM	28.9
ADF, % of DM	19.7
Soluble protein, % of CP	22.6
Neutral detergent-insoluble CP, % of DM	2.42
Acid detergent-insoluble CP, % of DM	0.72
Gross energy, Mcal/kg of DM	4.31

¹Approximately 70% red clover and 30% timothy. Dry matter content: 35.2%; chemical composition of the silage on a DM basis: 90.7% OM, 17.6% CP, 41.6% NDF, 30.8% ADF, 1.06% Ca, and 0.24% P.

²Contained on a DM basis: 70.1% ground shelled corn, 8.7% soybean meal 49%, 3.6% corn gluten meal, 10.7% wheat middlings, 2.5% expeller soybean meal, 1.64% mineral and vitamin mix, 0.55% calcium phosphate, 1% molasses, 0.23% salt, 0.46% calcium carbonate, 0.22% magnesium oxide, 0.17% vitamin E and selenium mix, and 0.11% Maxi-Bond (AgResearch, Joliet, IL). Chemical composition of the concentrate on a DM basis: 93.8% OM, 17.5% CP, 13.9% NDF, 3.7% ADF, 0.44% Ca, and 0.54% P.

on Animal Care (1993), and all procedures involving animals were previously approved by the Institutional Animal Care Committee of the Dairy and Swine Research and Development Center, Sherbrooke, Québec (Canada).

Cows and Diets

Six lactating multiparous Holstein cows fitted with ruminal cannulas (10 cm, Bar Diamond Inc., Parma, ID) were used in a double 3 × 3 Latin square design balanced for residual effects with 35-d periods. Cows averaged 90 ± 30 DIM and 704 ± 58 kg of BW at the start of the experiment. Cows were blocked by square according to milk production at the beginning of the study into high-producing (43.1 ± 6.4 kg/d) and low-producing cows (33.7 ± 2.0 kg/d). Before the study, milk fat and protein content averaged 3.17 ± 0.83% and 3.12 ± 0.17%, respectively. Cows were fed ad libitum a TMR (Table 1) without supplementation (control) or supplemented with MON or LAS at 24 mg/kg of DM, the highest level of MON licensed for use in dairy cattle in Canada (Canadian Food Inspection Agency, 2007). The IOP were mixed with the TMR as diluted pelleted pre-mixes: 2,000 and 4,000 mg/kg of DM for MON and LAS, respectively. Cows were housed in individual tie stalls and had free access to water throughout the experiment. Adaptation to experimental diets took place from d 1 to 26, milk sampling and total collection of feces and urine from d 27 to 31, and ruminal and blood sam-

pling on d 32. From d 33 to 35, an in situ experiment was conducted and results will be published later. Ionophores were introduced at 12 mg/kg of DM from d 1 to 5 and fed at 24 mg/kg of DM from d 5 until the end of each experimental period.

Diets were offered once daily at 0800 h and feed consumption was recorded daily. Daily feed allotment was calculated to allow 5 to 10% feed refusals. During the experiment, samples of TMR, feed ingredients, and orts were collected daily and frozen at -20°C. From d 27 to 31 of each period, the samples were pooled by period, freeze-dried, ground through a 1-mm screen Wiley mill (standard model 4, Arthur H. Thomas, Philadelphia, PA), and analyzed for DM, ash, total N, NDF, ADF, and gross energy (GE). Fresh silage extracts were prepared by mixing 20 g of freshly chopped silage in 50 mL of distilled water and macerating this for 2 h at room temperature. The extracts were filtered and the pH was determined (Accumet pH meter model 815 MP, Fisher Scientific, Fairlawn, NJ). The filtrate was then frozen at -20°C for later determination of NH₃-N and VFA concentrations. Body weight was determined at the beginning and the end of each experimental period after the a.m. milking on 2 consecutive days.

Apparent Total Tract Digestibility and N Balance

From d 27 to 31, urine was collected in stainless-steel containers via a Gooch tube (BF Goodrich Co., Kitchener, Ontario, Canada) and was acidified with H₂SO₄ (50% vol/vol) to maintain a pH <3.0. A representative sample (2%) was taken and kept frozen at -20°C for analyses of total N, urea-N, purine derivatives (PD), creatinine, and GE. Feces were collected during the same period in preweighed plastic-lined plywood boxes and were mixed daily. A representative sample (2%) was taken, stored at -20°C, and subsequently thawed, freeze-dried, and ground through a 1-mm screen (Wiley mill) for chemical analysis.

Milk Production and Milk Composition

Cows were milked twice daily in their stalls at 0800 and 1830 h, and milk yield was recorded at each milking. Milk samples were collected from each cow at each milking from d 27 (p.m.) to d 32 (a.m.). Milk subsamples from each milking were preserved with 2-bromo-2-nitropropan-1,3-diol, frozen at -20°C, and sent in a batch at the end of the experiment to a commercial laboratory (Programme d'Analyse des Troupeaux Laitiers du Québec, Ste-Anne-de-Bellevue, Québec, Canada) for infrared evaluation of fat, protein, lactose, and MUN content. Milk subsamples without preservative were pooled on a yield basis and kept frozen at -20°C until analyzed for milk fatty acid (FA) profile.

Rumen Fermentation Characteristics and Blood Metabolites

On d 32, ruminal content was sampled at 0, 1, 2, 3, 4, 5, and 6 h after the 0800 h feeding. Ruminal samples were taken from the ventral sac and strained through 2 layers of cheesecloth. Ruminal fluid pH was measured immediately after sampling (Piccolo pH meter, Hanna Instruments, Woonsocket, RI) and subsamples were acidified to pH 2 with sulfuric acid (50% vol/vol; 1:17 wt/wt acid:rumen fluid) and frozen at -20°C for later determination of VFA and $\text{NH}_3\text{-N}$ concentrations.

On d 32, blood was collected from the coccygeal vein at 0, 1, 2, 3, 4, 5, and 6 h after the morning feeding in Vacutainer tubes containing heparin (Becton Dickinson, Rutherford, NJ) and at 4 h in Vacutainer tubes without anticoagulant (Becton Dickinson, Franklin Lakes, NJ). The plasma was immediately separated by centrifugation ($2,000 \times g$, 12 min, 4°C) and an aliquot from each collection time was kept frozen at -20°C for the determination of urea-N concentration. Concentrations of plasma AA were determined in 3 pooled samples prepared as follows: 1 and 2 h; 3 and 4 h; and 5 and 6 h. An internal standard solution of AA labeled with stable isotopes (Martineau et al., 2007) was added to the pooled plasma and processed samples were frozen at -80°C . Blood without anticoagulant was allowed to clot for 1 h at room temperature and was then centrifuged at $2,000 \times g$ for 12 min at 4°C . All serum samples were free of hemolysis and were frozen at -20°C until analyzed for serum metabolites at Faculté de Médecine Vétérinaire, Université de Montréal (St-Hyacinthe, Québec, Canada).

Chemical Analyses

Analytical DM content was determined by oven-drying at 135°C for 2 h, followed by hot weighing (method 3.002; AOAC, 1990). Subsamples were ashed at 550°C for 12 h in a muffle furnace (method 942.05; AOAC, 1990), and OM content was determined as the difference between 100 and the percentage of ash. Total N concentration was determined by combustion (Leco model FP-428 Nitrogen Determinator, Leco, St. Joseph, MI) and CP was calculated as $\text{N} \times 6.25$. Feed samples were analyzed for CP soluble in borate-phosphate buffer according to Roe et al. (1990). The concentration of NDF was determined as described by Van Soest et al. (1991), using sodium sulfite and heat-stable α -amylase. The ADF content was determined as described by Robertson and Van Soest (1981). The NDF and ADF procedures were adapted for use in an Ankom²⁰⁰ fiber analyzer (Ankom Technology, Fairport, NY). Gross energy was determined with an adiabatic bomb calorimeter (model 1241, Parr Instrument Co., Moline, IL).

Total N concentration in urine, insoluble residues from the borate-phosphate procedure, and NDF and ADF residues was determined by micro-Kjeldahl analysis (method 955.04; AOAC, 1990). Purine derivatives and creatinine concentrations in urine were determined according to Balcells et al. (1992) by using a Beckman System Gold chromatograph (Beckman Instruments, Fullerton, CA). Concentration of $\text{NH}_3\text{-N}$ in ruminal fluid was determined by using the indophenol-blue method (Novozamsky et al., 1974). Concentration of VFA in ruminal fluid was measured with an HPLC Gold System (Beckman Instruments, San Ramon, CA).

Protein, fat, lactose, TS, and urea-N concentrations in milk samples were analyzed by infrared spectroscopy (System 4000 MilkoScan, Foss Electric, Hillerød, Denmark). Milk N was calculated as protein concentration divided by 6.38. Fatty acids in milk were extracted, methylated, and prepared according to the methods previously used by Petit (2002). Fatty acid methyl ester profiles were measured by GLC (Hewlett-Packard 6890 chromatograph, Hewlett-Packard Ltd., Montréal, Québec, Canada) with a G1315A autosampler equipped with a flame-ionization detector and a split-splitless injector, as described by Delbecchi et al. (2001).

Urea-N concentrations in plasma and urine were determined colorimetrically by using a Technicon autoanalyzer (Technicon Instruments, Tarrytown, NY; Huntington, 1984). Plasma AA concentrations were determined by isotopic dilution with a gas chromatograph-mass spectrometer (model GC6890-MS973, Agilent Technologies, Wilmington, DE) as described by Calder et al. (1999), and results were pooled by cow and period. Serum metabolites were determined by using an automated autoanalyzer (Synchron CX5 Clinical System, Fullerton, CA), except for selenium, which was measured by a chromatograph (HP 1100 HPLC, Hewlett-Packard, Palo Alto, CA; Hawkes and Kutnink, 1996).

Calculations and Statistical Analyses

Rumen microbial N (MN) outflow was determined according to the equations of Chen and Gomes (1992):

$$Y \text{ (mmol/d)} = (0.85 \times X) + (0.385 \times \text{BW}^{0.75}), \text{ and}$$

$$\text{MN (g/d)} = [70 / (0.116 \times 0.83 \times 1,000)] \times X,$$

where X and Y are the amounts of absorbed and excreted PD (mmol/d), respectively. Those equations assume that the daily excretion of PD in milk is 1 mmol/kg of milk produced, the N content of purines is 70 mg/mmol, the ratio of purine N to total N in mixed rumen microbes is 0.116, the endogenous contribution to urinary PD excretion is $0.385 \times \text{BW}^{0.75}$ mmol/d, the true

Table 2. Effects of lasalocid (LAS) or monensin (MON) on DMI and BW change of lactating dairy cows

Item	Treatment ¹			SEM	Contrast ²	
	Control	LAS	MON		Control vs. IOP	LAS vs. MON
DMI						
kg/d	23.1	24.0	23.4	0.5	0.27	0.32
% of BW	3.07	3.21	3.11	0.10	0.30	0.37
BW						
Initial, kg	750	742	752	24	0.76	0.39
Final, kg	762	760	760	21	0.75	0.97
Change, kg/d	0.36	0.50	0.30	0.23	0.81	0.37

¹Least squares means with SEM given for n = 5.

²P-value for contrasts: control vs. ionophores (IOP) and LAS vs. MON.

intestinal digestibility of microbial purines is 83%, and the recovery of absorbed purines as urinary PD is 85%.

Data were analyzed by using PROC MIXED (SAS Institute, 2000) according to the model

$$Y_{ijkl} = \mu + S_i + P_j + C_{k(i)} + T_l + ST_{il} + \varepsilon_{ijkl},$$

where Y_{ijkl} is the response variable, μ is the overall mean, S_i is the effect of square i , P_j is the effect of period j , $C_{k(i)}$ is the random effect of cow k (within square i), T_l is the effect of treatment l , ST_{il} is the interaction between square i and treatment l , and ε_{ijkl} is the residual error. Sampling time and the interaction between sampling time and treatment were added to the model for the analysis of ruminal fermentation characteristics and plasma urea-N, and were analyzed as repeated measures by using PROC MIXED. Autoregressive order 1 and compound symmetry (homogenous and heterogenous) were tested as covariance structures, and the covariance structure with the least Akaike information criterion was retained in the final model. The interaction term ST_{il} was removed when $P > 0.25$. Sums of squares for treatment were separated into single degree of freedom preplanned orthogonal contrasts: control vs. ionophores and LAS vs. MON. Results are reported as least squares means \pm standard error of the means. Significance was declared at $P \leq 0.05$ and a trend at $P \leq 0.10$. Results are also reported for each square when the interaction term ST_{il} was significant ($P \leq 0.05$) or when a tendency or a significant effect was detected when squares were analyzed separately. Data from one cow during the last period were deleted because of a health problem (hock bursitis) unrelated to treatments.

RESULTS AND DISCUSSION

Intake and BW

The intake of both IOP averaged 565 mg/d, or 24 mg/kg of DM. Dry matter intake and changes in BW were unaffected ($P > 0.30$) by treatments (Table 2). Similarly,

Beede et al. (1986) and Ali Haïmoud et al. (1995) observed no change in DMI when lactating cows were fed 12 or 24 mg/kg of DM of LAS or 33 mg/kg of DM of MON. Conversely, Johnson et al. (1988) and Cant et al. (1997) observed a reduction in DMI of lactating cows fed LAS (36.7 mg/kg of DM) or MON (14.5 mg/kg of DM). Symanowski et al. (1999) examined the effects of MON supplementation involving 858 Holstein cows and reported that MON reduced DMI by 4% over the entire 305-d lactation period when supplemented at 16 to 24 mg/kg of DM compared with 0 to 8 mg/kg of DM. A further analysis of those data by Wagner et al. (1999) suggested variable responses to MON supplementation over the lactation period: DMI was not reduced in early-lactating cows, whereas it was decreased when cows were in mid- or late lactation. According to Tedeschi et al. (2003), the additional energy available because of MON is used to improve performance, reduce body reserve losses, or both when cows are in early lactation, whereas MON inclusion in late lactation results in lower DMI because cows are eating to their energy requirement.

Ruminal Fermentation Characteristics and Plasma Urea-N

There was a square by treatment interaction for ruminal pH ($P < 0.01$; Figure 1). In low-producing cows, ruminal pH tended ($P = 0.08$) to be lower with LAS than with MON supplementation (6.00 vs. 6.16), whereas in high-producing cows, ruminal pH was lower ($P = 0.02$) with LAS than with MON supplementation (5.74 vs. 5.99). The square by treatment interaction on milk fat concentration was not significant, yet milk fat concentration tended ($P = 0.09$) to be lower with LAS than MON (3.18 vs. 3.40%) in high-producing cows, suggesting a link between the low rumen pH and the low milk fat concentration observed with LAS.

Total VFA concentration was higher ($P = 0.05$) with LAS than with MON supplementation (Table 3). An

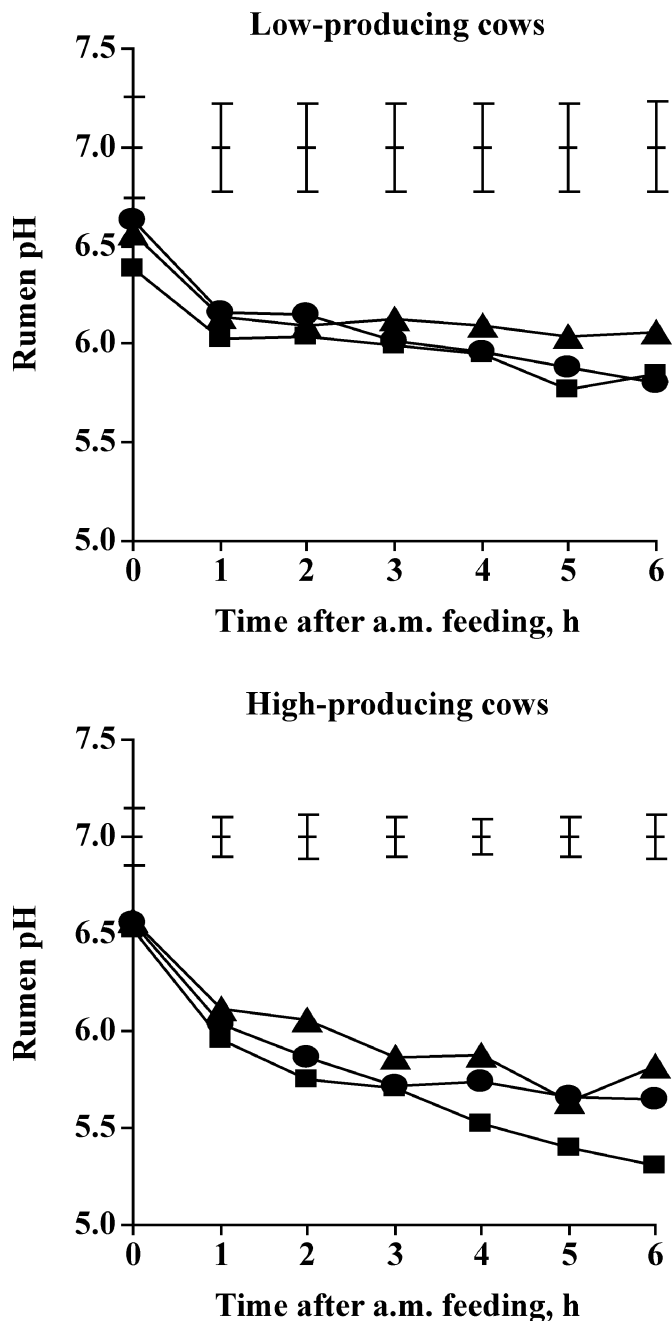


Figure 1. Least squares mean values for rumen pH after the morning feeding (0800 h) in low- and high-producing midlactating Holstein cows fed monensin (▲) or lasalocid (■) in the diet at 24 mg/kg, or that served as negative controls (●). Error bars indicate SEM, and the square by treatment interaction was significant ($P < 0.01$).

increased rumen digestion of OM or starch could be responsible for the higher concentration of rumen VFA and the lower pH with LAS compared with MON. In feedlot cattle, Zinn (1987) observed that OM and starch digestion in the rumen were numerically greater with LAS compared with MON. However, in that study, ru-

men digestion of OM and starch could have been biased negatively during the MON treatment because of the inclusion of tylosin with MON. Even though the proportions of individual VFA were unaffected by treatments, the lipogenic:glucogenic VFA ratio was lower ($P = 0.05$) with IOP supplementation, as previously reported by Johnson et al. (1988) with LAS (37 mg/kg of DM) and Sauer et al. (1989) with MON (15 to 30 mg/kg of DM).

Supplementation with IOP tended ($P = 0.07$) to decrease ruminal $\text{NH}_3\text{-N}$ concentration (Table 3 and Figure 2). Monensin supplementation has been reported to inhibit growth and activity of the proteolytic and obligate AA-fermenting bacteria (Yang and Russell, 1993), thus decreasing AA deamination and the rate of $\text{NH}_3\text{-N}$ production in the rumen. No difference in ruminal $\text{NH}_3\text{-N}$ concentration was obtained between MON and LAS, which agrees with the findings of Guan et al. (2006), who observed that a rotation of MON (33 mg/kg of DM) and LAS (36 mg/kg of DM) in steers resulted in a similar reduction of ruminal $\text{NH}_3\text{-N}$ concentration.

In theory, the pattern of plasma urea-N concentration over time should reflect the ruminal $\text{NH}_3\text{-N}$ concentration because the liver essentially removes all net portal absorption of $\text{NH}_3\text{-N}$, which can account for 70 to 80% of urea-N released by the liver when hepatic function is not impaired (Huntington, 1990). Similar patterns for both ruminal $\text{NH}_3\text{-N}$ (Figure 2) and plasma urea-N (Figure 3) concentrations were obtained in the present study, with peak concentrations of plasma urea-N lagging about 1 h behind the peak in rumen $\text{NH}_3\text{-N}$. However, there was a clear disparity between these variables. Plasma urea-N concentrations were 11.5, 10.7, and 12.7 mg/dL (SEM 0.7) for the control, LAS, and MON, respectively. Plasma urea-N concentration was markedly higher ($P = 0.05$) for MON than LAS, as was serum urea-N concentration (10.1 vs. 8.2 mM; $P < 0.01$) in blood sampled 4 h after the morning feeding.

It is paradoxical to observe an increase in plasma urea-N when ruminal $\text{NH}_3\text{-N}$ is decreased, yet this effect has been reported previously in lambs (Poos et al., 1979) and in finishing steers (Thompson and Riley, 1980). The increase in plasma urea-N could result from an inhibition of urea recycling into the gastrointestinal tract (Harmon et al., 1989). To our knowledge, no study has reported this paradoxical effect in dairy cows supplemented with IOP, although increased serum urea or blood urea-N concentrations were previously observed in peripartum dairy cows supplemented with 335 mg/d of MON (Duffield et al., 1998) or 350 mg/d of LAS (McDougall et al., 2004). Duffield et al. (1998) attributed this effect to increased absorption and catabolism of nonessential AA to meet the high energy demand of cows in early lactation. This is obviously not

Table 3. Effects of lasalocid (LAS) or monensin (MON) on ruminal fermentation in lactating dairy cows

Item	Treatment ¹			SEM	Contrast ²	
	Control	LAS	MON		Control vs. IOP	LAS vs. MON
Total VFA, mM	137	143	132	8	0.84	0.05
VFA, mol/100 mol						
Acetate (A)	60.2	59.2	60.5	1.5	0.82	0.40
Propionate (P)	24.6	25.9	25.8	1.2	0.15	0.92
Butyrate (B)	12.2	12.1	10.7	0.7	0.27	0.11
(A + B):P ratio	3.10	2.83	2.77	0.21	0.05	0.68
NH ₃ -N, mg/dL	9.3	6.8	7.7	1.3	0.07	0.51

¹Least squares means with SEM given for n = 5.

²P-value for contrasts: control vs. ionophores (IOP) and LAS vs. MON.

the case in the present experiment because there was no difference in milk production and composition. Duffield et al. (1998) also suggested that the improved energy status with MON might reduce fatty infiltration of liver, thus improving its function and enhancing its ability to synthesize urea. Again, this is probably not the case in this study because cows were gaining weight and apparently not mobilizing body fat.

Apparent Total Tract Digestibility, N Balance, and MN Outflow

Supplementation with IOP increased ($P \leq 0.05$) apparent total tract digestibility of DM (**DMAD**), OM (**OMAD**), CP, and GE compared with the control, and there was no difference ($P \geq 0.20$) between LAS and MON (Table 4). This is contradictory to other studies

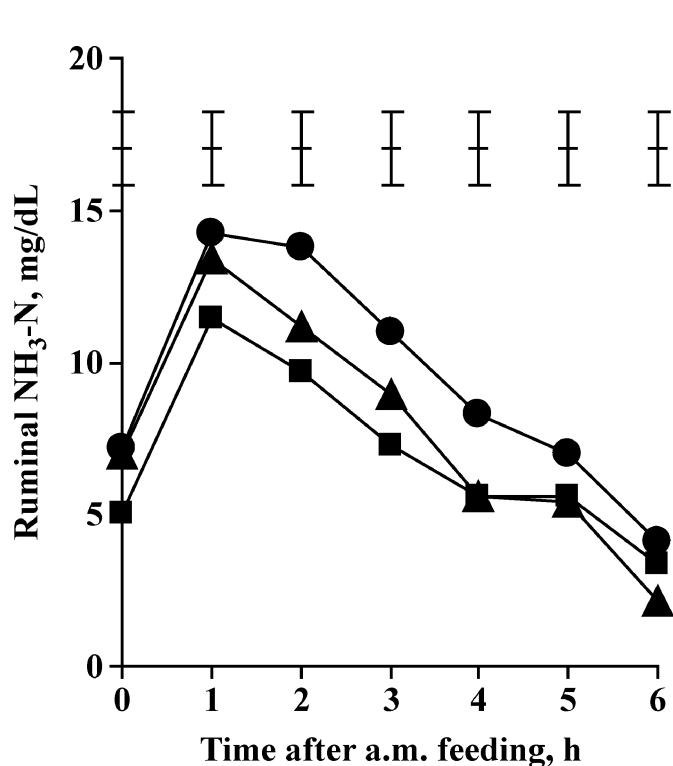


Figure 2. Least squares mean values for rumen NH₃-N concentration (mg/dL) after the morning feeding (0800 h) in midlactating Holstein cows fed monensin (▲) or lasalocid (■) in the diet at 24 mg/kg, or that served as negative controls (●). Error bars indicate SEM, and the square by treatment interaction was not significant.

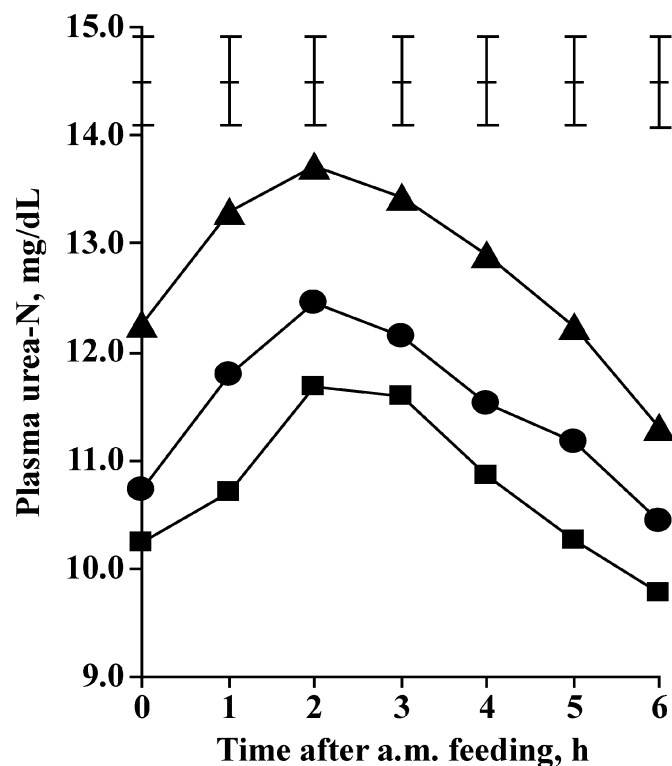


Figure 3. Least squares mean values for plasma urea-N concentration (mg/dL) after the morning feeding (0800 h) in midlactating Holstein cows fed monensin (▲) or lasalocid (■) in the diet at 24 mg/kg, or that served as negative controls (●). Error bars indicate SEM, and the square by treatment interaction was not significant.

Table 4. Effects of lasalocid (LAS) or monensin (MON) on apparent total tract digestibility, N balance, urinary excretion of urea N, and rumen microbial N outflow in lactating dairy cows

Item	Treatment ¹			SEM	Contrast ²	
	Control	LAS	MON		Control vs. IOP	LAS vs. MON
Digestibility, %						
DM	71.4	73.8	72.7	0.9	0.03	0.21
OM	72.5	74.7	74.0	0.9	0.02	0.38
CP	64.4	67.2	66.4	1.4	0.05	0.53
NDF	53.8	54.5	54.6	2.4	0.70	0.96
ADF	54.5	54.3	53.7	3.0	0.85	0.86
Gross energy	70.6	72.7	72.0	0.9	0.02	0.36
N balance, g/d						
Intake	655	675	654	17	0.55	0.26
Feces	233	221	219	7	0.08	0.77
Urine	165	147	183	8	0.99	<0.01
Milk	187	195	193	7	0.32	0.85
Retained	70	112	60	12	0.27	0.01
Urinary urea-N, g/d	128	112	143	6	0.83	<0.01
Microbial N outflow, ³ g/d	322	252	299	27	0.17	0.23

¹Least squares means with SEM given for n = 5.

²P-value for contrasts: control vs. ionophores (IOP) and LAS vs. MON.

³Estimated from the urinary excretion of purine derivatives according to the equations of Chen and Gomes (1992).

in which MON had no effect on DMAD and OMAD (Ali-Haïmoud et al., 1995; Plaizier et al., 2000; MacKintosh et al., 2002) and in which LAS had no effect on DMAD (Dye et al., 1988; Knowlton et al., 1996).

The effect of IOP supplementation on CP apparent digestibility appears less variable than its effect on DMAD or OMAD. Significant increases in CP apparent digestibility have been reported when dairy cattle were fed MON (Plaizier et al., 2000; Ruiz et al., 2001; Benchaar et al., 2006). On the other hand, Dye et al. (1988) reported a linear increase in CP apparent digestibility with increasing LAS dosage rates up to 19 mg/kg of DM.

The improved CP digestibility observed with IOP could result from a higher ratio of dietary to microbial CP entering the small intestine because dietary CP can be more digestible than microbial CP (Spears, 1990). In our study, RUP digestibility was estimated to be higher than microbial CP digestibility. Using NRC (2001) predictions for passage and intestinal digestibility of RUP for each feed ingredient, we estimated RUP digestibility of the diet to be between 86 and 88% depending on maturity at harvest. On the other hand, MN intestinal digestibility was estimated at 74%, assuming that 80% of MN is true protein N with 80% intestinal digestibility (NRC, 2001), 11.6% is nucleic acid N with 83% intestinal digestibility (Chen and Gomes, 1992), and the rest is indigestible cell wall peptidoglycan N. In our study, MN passage to the small intestine was numerically lower (322 vs. 275 g/d; $P = 0.17$; Table 4) when supplementing the ration with IOP, and other researchers have reported a significant decrease in MN

passage to the small intestine when feeding MON (Poos et al., 1979; Hoeller et al., 1985). Spears (1990) also speculated that metabolic fecal protein losses might be reduced by IOP feeding, and this may also partially explain the higher apparent CP digestibility observed when supplying IOP to the ration.

Intake of N was similar among treatments (Table 4). Fecal excretion of N tended ($P = 0.08$) to be lower for IOP-supplemented diets than for the control, whereas N secretion in milk was unaffected by IOP supplementation. The amounts of N excreted in feces and milk were similar for cows fed MON and LAS. However, urinary N excretion was lower ($P < 0.01$) for LAS than for MON, which resulted in higher ($P < 0.01$) N retention in cows fed LAS. Few studies have reported the effect of MON on N balance in dairy cows (Plaizier et al., 2000; Ruiz et al., 2001; Benchaar et al., 2006) and, to our knowledge, no study has reported the effect of LAS on N balance in dairy cows. Plaizier et al. (2000) and Ruiz et al. (2001) reported lower fecal N excretion with MON compared with the control, and Plaizier et al. (2000) observed a concomitant increase in N retention with MON supplementation. On the other hand, Benchaar et al. (2006) reported no effect of feeding MON (16 mg/kg of DM) on N balance (g/d).

The proportion of total urinary N as urea-N averaged 77% and was unaffected by treatments (SEM 1; $P = 0.52$). However, LAS decreased ($P < 0.01$) urea-N excretion in urine compared with MON, in agreement with the lower concentrations of plasma urea-N and milk urea-N. In high-producing cows, MN duodenal flow as

Table 5. Effects of lasalocid (LAS) or monensin (MON) on the milk production and milk composition of dairy cows

Item	Treatment ¹			SEM	Contrast ²	
	Control	LAS	MON		Control vs. IOP	LAS vs. MON
Milk production, kg/d	35.6	37.3	36.9	2.0	0.26	0.83
4% FCM, ³ kg/d	31.5	33.4	33.5	1.6	0.16	0.94
Milk production efficiency, kg of milk/kg of DMI	1.39	1.41	1.45	0.06	0.42	0.58
Milk composition, %						
Fat	3.29	3.37	3.41	0.24	0.53	0.83
Protein	3.40	3.36	3.37	0.11	0.28	0.64
Lactose	4.59	4.70	4.60	0.07	0.28	0.18
TS	11.28	11.42	11.39	0.28	0.38	0.82
Milk yield, kg/d						
Fat	1.15	1.23	1.25	0.08	0.17	0.84
Protein	1.19	1.24	1.23	0.04	0.32	0.85
Lactose	1.64	1.75	1.71	0.12	0.26	0.65
TS	3.98	4.23	4.19	0.19	0.19	0.83
Milk urea-N, mg/dL	11.7	11.0	12.1	1.0	0.67	0.02

¹Least squares means with SEM given for n = 5.

²P-value for contrasts: control vs. ionophores (IOP) and LAS vs. MON.

³4% FCM = 0.4 × (milk, kg/d) + 15.0 × (fat, kg/d).

estimated with PD was 305, 191, and 283 g/d (SEM 32) for the control, LAS, and MON, respectively. The difference between LAS and MON did not reach significance ($P = 0.18$) but might indicate that microbial synthesis was disturbed by acidotic rumen pH. In low-producing cows, MN duodenal flow was 338, 312, and 298 g/d (SEM 53; $P = 0.84$) for the control, LAS, and MON, respectively. In agreement with our results, Ali Haimoud et al. (1996) reported that passage of bacterial N to the small intestine was not affected by feeding avoparcin or MON at 33 mg/kg of DM. However, other studies showed that both LAS (Thivend and Jouany, 1983; Gomez et al., 1991) and MON (Poos et al., 1979; Hoeller et al., 1985) could exert an antimicrobial action in the rumen, thus decreasing MN outflow to the duodenum.

Milk Production and Milk Composition

Milk production averaged 42.4 kg/d (SEM 3.5) and 30.2 kg/d (SEM 1.5) for the high- and low-producing cows, respectively. In low-producing cows, milk production tended ($P = 0.08$) to be higher with IOP (30.5 vs. 29.6 kg/d; SEM 1.5) and was higher ($P = 0.05$) for LAS than MON (31.6 vs. 29.4 kg/d), despite a similar DMI among treatments (22.8 kg of DM/d; SEM 0.9; $P = 0.90$).

Monensin supplementation usually increases milk production and decreases milk fat and protein percentages (Hayes et al., 1996; Symanowski et al., 1999; Phipps et al., 2000). The response in milk production to LAS supplementation is less clear. Feeding LAS reduced milk production of cows in the study of Johnson et al.

(1988), whereas it gave different results depending on whether LAS was fed to primiparous (increased milk production) or multiparous cows (decreased milk production) in the study by Knowlton et al. (1996). Only 2 studies were found in which a significant effect on milk components was reported with LAS supplementation. Dye et al. (1988) reported a linear reduction in milk fat concentration but no change in milk protein concentration in cows fed increased amounts of LAS (6 to 19 mg/kg of DM). Knowlton et al. (1996) observed a decrease in milk fat content and an increase in milk protein content in cows supplemented with 18 mg/kg of DM of LAS. More studies are required to clearly establish the effect of LAS supplementation on milk production and composition.

Milk urea-N concentration was lower ($P = 0.02$; Table 5) with LAS compared with MON, in line with the effects on plasma urea-N and urinary urea-N excretion (Table 4). Urea diffuses passively from blood to milk (Gustafsson and Palmquist, 1993) and a linear relationship exists between urinary N excretion or plasma urea-N and MUN concentrations (Kauffman and St-Pierre, 2001).

There was a significant square by treatment interaction for relative percentages (% of total FA) of 18:3 and polyunsaturated FA (PUFA) in milk. In high-producing cows, relative percentages of 18:3 were 1.11, 1.70, and 1.37% (SEM 0.08) for the control, LAS, and MON, respectively. The percentage of 18:3 increased ($P = 0.05$) with IOP supplementation and tended ($P = 0.10$) to be greater for LAS than for MON. In low-producing cows, the proportions of 18:3 were 1.51, 1.27, and 1.48% (SEM

Table 6. Effects of lasalocid (LAS) or monensin (MON) on the fatty acid profile of milk fat of dairy cows

Item	Treatment ¹			SEM	Contrast ²	
	Control	LAS	MON		Control vs. IOP	LAS vs. MON
10:0	4.7	4.4	4.3	0.2	0.02	0.37
12:0	5.9	5.6	5.5	0.3	0.04	0.61
14:0	15.3	15.0	14.9	0.4	0.10	0.92
14:1	1.53	1.56	1.66	0.16	0.31	0.28
16:0	34.2	35.2	34.8	1.4	0.07	0.34
16:1	2.16	2.22	2.47	0.20	0.03	0.02
18:0	8.3	8.2	7.8	0.4	0.25	0.28
<i>Trans</i> -18:1	1.69	1.72	1.64	0.10	0.80	0.26
<i>Cis</i> -18:1	19.8	19.9	20.5	0.7	0.36	0.28
18:2 (total) ³	4.76	4.50	4.73	0.30	0.15	0.09
<i>Cis</i> -9, <i>trans</i> -11 18:2	0.66	0.67	0.63	0.05	0.92	0.25
18:3 ⁴	1.29	1.47	1.31	0.11	0.38	0.25
Other ³	0.41	0.42	0.41	0.04	0.97	0.75
MCFA ³	53.2	53.9	53.8	1.2	0.27	0.89
LCFA ³	36.2	36.1	36.4	1.2	0.88	0.69
Saturated ³	68.4	68.2	67.4	1.1	0.21	0.14
MUFA ³	25.1	25.4	26.2	0.8	0.13	0.10
PUFA ^{3,4}	6.48	6.40	6.52	0.39	0.88	0.52

¹Least squares means with SEM given for n = 5.

²P-value for contrasts: control vs. ionophores (IOP) and LAS vs. MON.

³18:2 (total) = *cis*-10, *cis*-12 18:2 + *cis*-10, *trans*-12 18:2 + *cis*-9, *trans*-11 18:2; other = 20:3 + 20:4 + 20:5 + 22:6; MCFA = medium-chain fatty acids (14:0 to <18:0); MUFA = monounsaturated fatty acids (14:1 + 16:1 + 18:1); LCFA = long-chain fatty acids (>16:0); saturated = 10:0 + 12:0 + 14:0 + 16:0 + 18:0; PUFA = polyunsaturated fatty acids (18:2 + 18:3 + other).

⁴Square by treatment interaction ($P \leq 0.05$).

0.23; $P = 0.37$) for the control, LAS, and MON, respectively. In high-producing cows, relative percentages of PUFA were 6.14, 6.60, and 6.42% (SEM 0.44) for the control, LAS, and MON, respectively, and they increased ($P = 0.03$) with IOP supplementation, but there was no difference ($P = 0.12$) between LAS and MON. In low-producing cows, relative percentages of PUFA were not affected by treatments, being 6.83, 6.21, and 6.69% (SEM 0.67; $P = 0.32$) for the control, LAS, and MON, respectively.

Ionophore supplementation tended ($P \leq 0.10$) to decrease the relative percentages of 10:0, 12:0, and 14:0 in milk, whereas it tended ($P \leq 0.07$) to increase those of 16:0 and 16:1 (Table 6). Lasalocid decreased ($P \leq 0.10$) milk relative percentages of 16:1, total 18:2, and MUFA compared with MON. Supplementation with IOP had no effect on relative percentages of 14:1, 18:0, *trans*-18:1, *cis*-18:1, *cis*-9, *trans*-11 18:2, other FA, medium- and long-chain FA, and saturated FA in milk. Milk short-chain FA in the mammary gland are derived mainly from de novo synthesis by using circulating acetate and BHBA originating from the rumen (Pethick and Dunshea, 1993). Therefore, lower relative percentages of short-chain FA agree with the negative effect of IOP on the lipogenic:glucogenic ruminal VFA ratio (Table 3). The trend ($P = 0.07$) for higher 16:0 in the milk fat of cows fed IOP might be associated with the

greater ($P = 0.03$) concentrations of serum NEFA (Table 7), because mammary gland uptake of preformed FA from the digestive tract or mobilized from body fat as NEFA is important for the synthesis of 16:0 and all long-chain FA (Pethick and Dunshea, 1993).

Serum Metabolites, Liver Enzymes, and Plasma AA

There were no effects of treatments ($P > 0.20$) on serum concentrations (mean \pm SE) of glucose (3.36 \pm 0.10 mM), cholesterol (4.9 \pm 0.4 mM), albumin (35.2 \pm 0.6 g/L), globulin (39.5 \pm 2.3 g/L), total protein (74.7 \pm 2.5 g/L), calcium (2.39 \pm 0.05 mM), phosphorus (1.97 \pm 0.14 mM), magnesium (0.97 \pm 0.02 mM), potassium (4.3 \pm 0.2 mM), sodium (139.0 \pm 0.9 mM), chloride (100.9 \pm 0.9 mM), copper (16.1 \pm 0.8 μ M), β -carotene (2.1 \pm 0.1 μ M), or osmotic pressure (286 \pm 2 mosm/L).

Current results on the effect of treatments on serum metabolites should be interpreted with caution because values remained within or close to normal-range values for midlactating cows (Table 7). Furthermore, deviations from normal-range values were observed in the control treatment for BHBA, NEFA, and liver enzymes. Supplementation with IOP tended ($P = 0.06$) to decrease serum selenium concentration, whereas it increased ($P = 0.05$) glutathione peroxidase activity, an indirect indicator of oxidative stress (Tüzün et al., 2002). In-

Table 7. Effects of lasalocid (LAS) or monensin (MON) on serum metabolites

Item ¹	Treatment ²			SEM	Contrast ³	
	Control	LAS	MON		Control vs. IOP	LAS vs. MON
Urea-N, mM	9.2	8.2	10.1	0.5	0.91	<0.01
BHBA, μ M	863	850	594	104	0.21	0.08
NEFA, μ M	149	169	163	8	0.03	0.44
Selenium, μ M	1.04	1.01	0.99	0.03	0.06	0.50
GSH-Px, units/L	325	370	357	21	0.05	0.52
GGT, units/L	35.3	34.8	37.5	2.1	0.33	0.04
AST, units/L	90.8	87.5	99.4	10.1	0.60	0.08
Bicarbonate, mM	27.1	29.0	30.2	0.6	0.01	0.19
Anion gap, mM	15.4	13.1	12.9	0.7	0.03	0.84

¹Normal-range values of serum metabolites for midlactating cows (Faculté de Médecine Vétérinaire, Université de Montréal, St-Hyacinthe, Québec, Canada) = urea-N (6.7–13.2 mM), BHBA (460–760 μ M), NEFA (190–425 μ M), selenium (1.04–1.26 μ M), GSH-Px (glutathione peroxidase activity; 150–400 units/L), GGT (γ -glutamyl transpeptidase; 21–30 units/L), AST (aspartate aminotransferase; 51–67 units/L), bicarbonate (24.6–28.9 mM), anion gap (sodium and potassium minus chloride and bicarbonate; 14.5–19.8 mM).

²Least squares means with SEM given for n = 5.

³P-value for contrasts: control vs. ionophores (IOP) and LAS vs. MON.

trastesticular administration of MON (5 μ g/testis) to rats induced a marked increase in lipid peroxidation (Singh et al., 2006). Supplementation with IOP also induced a metabolic alkalosis, as shown by the greater ($P = 0.01$) concentration of serum bicarbonate associated with a lower ($P = 0.03$) anion gap value. The lower anion gap value could not be linked to any increase in serum concentrations of calcium, magnesium, or globulins or to a decrease in serum concentration of albumin.

Differences were observed between LAS and MON for serum concentrations of BHBA, γ -glutamyl transpeptidase (GGT), and aspartate aminotransferase (AST). Serum BHBA concentration tended ($P = 0.08$) to be greater for cows supplemented with LAS than for those fed MON. This effect is likely due to differences in the absorption and conversion of ruminal butyrate into BHBA by the ruminal epithelium (Baird et al., 1980), which corroborates the trend ($P = 0.06$) for higher

Table 8. Effects of lasalocid (LAS) or monensin (MON) on plasma concentrations of AA in lactating dairy cows

AA, μ M	Treatment ¹			SEM	Contrast ²	
	Control	LAS	MON		Control vs. IOP	LAS vs. MON
His	56.2	60.9	58.1	2.8	0.19	0.36
Ile	123.2	122.7	110.6	7.5	0.33	0.16
Leu	196.7	198.5	182.9	12.0	0.59	0.27
Lys	74.3	73.5	70.4	3.4	0.55	0.53
Met	17.4	18.9	16.8	0.9	0.59	0.08
Phe	49.7	50.8	47.4	1.4	0.70	0.11
Thr	96.9	111.1	94.4	8.1	0.39	0.07
Trp	60.6	60.2	59.2	2.1	0.67	0.71
Val	265.4	265.0	247.6	18.4	0.51	0.32
Ala	227.6	238.5	232.6	21.9	0.40	0.60
Gln	268.4	259.4	269.7	12.7	0.74	0.46
Glu	49.9	53.1	49.3	1.9	0.50	0.12
Gly	291.2	343.0	278.6	23.2	0.28	0.02
Ser	95.5	107.0	96.2	5.3	0.23	0.10
Tyr	61.9	63.9	61.3	3.1	0.81	0.48
Total AA	1,935	2,026	1,879	79	0.81	0.12
Essential AA	940	961	888	48	0.73	0.20
Nonessential AA	994	1,065	990	52	0.35	0.10
Branched-chain AA	585	586	541	37	0.48	0.24

¹Least squares means with SEM given for n = 5.

²P-value for contrasts: control vs. ionophores (IOP) and LAS vs. MON.

molar concentration of ruminal butyrate (17.6 vs. 14.4 mM; SEM 1.7) in cows fed LAS compared with cows supplemented with MON. Serum concentrations of GGT and AST tended ($P \leq 0.08$) to be greater for MON than for LAS (Table 7), suggesting that MON caused slightly more liver damage than LAS. In contrast to our results, MON decreased serum concentrations of GGT (Cécyre, 2001) and AST (Duffield et al., 1998) in early-lactating cows that received a controlled-release capsule containing MON 2 to 4 wk prior to calving compared with control cows.

Plasma concentrations of most AA were unaffected by IOP supplementation (Table 8). However, compared with MON, LAS tended ($P \leq 0.10$) to increase plasma concentrations of Met, Thr, Gly, Ser, and nonessential AA. Those 4 AA share common metabolic pathways, and the effect of IOP is intriguing because production data do not reflect a decreased utilization of these AA for neoglucogenesis or methylations.

CONCLUSIONS

In the current study, LAS and MON fed at 24 mg/kg of DM resulted in similar effects on N metabolism within the gastrointestinal tract. Both reduced ruminal $\text{NH}_3\text{-N}$ concentration and increased apparent CP digestibility. In contrast to LAS, however, MON increased concentrations of plasma urea-N and MUN, and increased excretion of urea-N in urine. This suggests a different effect on postabsorptive metabolism of N, which possibly includes a decreased recycling of urea-N into the gut. More research is needed to elucidate possible differences in the mechanisms of action of MON and LAS on N metabolism.

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